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INTEROFFICE CORRESPONDENCE
Richmond, Virginia

To: Distribution**Date:** November 14, 1997**From:** *W. McCoy, I. Skinner, W. Ryan, Jr.*
W. McCoy, I. Skinner, W. Ryan, Jr.**Subject:** Massachusetts Compliance.
CDC Protocol For Nicotine, Status Report III: Activity 7A14

This memo reports the nicotine levels in several cigarette fillers. The samples were prepared and the nicotine measured by the Centers for Disease Control and Prevention (CDC) protocol.¹

Sample Preparation

Initial sample preparation including conditioning, sampling and ripping of the cigarettes was done in the Product Testing Laboratories (PTL). The loose filler was placed in 60-oz plastic containers and transported to ARD Sample Room. After the samples were logged into the ARD LIMS, 100 grams of each sample were processed through the Robot Coupe RSI 6V as specified by the CDC protocol.¹ The processed filler samples were then each transferred into individual quart glass jars with screw caps.

Recoveries

Recoveries were determined in triplicate for each processed filler sample by the procedure specified in the CDC protocol.¹ Recovery factors for Request Codes MA973 and MA974 are reported in Tables 1 and 2, respectively.

Table 1: Recovery Data for Request Code MA973		
<u>Sample Code</u>	<u>Recovery Factor*</u>	
	<u>Individual Replicates</u>	<u>Average</u>
MA973-1	0.885	0.890
	0.895	
	0.890	
MA973-2	0.875	0.877
	0.880	
	0.875	
MA973-3	0.865	0.865
	0.865	
	0.865	
MA973-4	0.835	0.835
	0.835	
	0.835	

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Table 2:
Recovery Data for Request Code MA974

<u>Sample Code</u>	<u>Recovery Factor*</u>	
	<u>Individual Replicates</u>	<u>Average</u>
MA974-1	0.920	0.920
	0.920	
	0.920	
MA974-2	0.925	0.925
	0.925	
	0.925	
MA974-3	0.935	0.933
	0.930	
	0.935	
MA974-4	0.915	0.912
	0.910	
	0.910	

Nicotine Analyses

Nicotine levels for each of the processed filler samples were determined in triplicate by the procedure specified in the CDC protocol.¹ The results for sample groups 3 and 4 are reported in Tables 3 and 4, respectively. The individual replicates for each sample are indicated by the -A, -B and -C suffixes appended to the sample code.

Table 3: Nicotine Data for Request Code MA973

<u>Sample Code</u>	<u>Wt % Nicotine</u>			<u>Average Weight (g) of Filler per Cigarette</u>	<u>Mg Nicotine per cigarette</u>
	<u>Not Corrected for Recovery</u>	<u>Corrected for Recovery*</u>	<u>Average Recovery Factor*</u>		
MA973-1-A	1.69	1.90	0.890	0.753	14.27
-B	1.61	1.81			13.59
-C	1.60	1.80			13.56
MA973-2-A	1.64	1.87	0.877	0.717	13.37
-B	1.63	1.86			13.32
-C	1.61	1.84			13.16
MA973-3-A	1.64	1.90	0.865	0.674	12.80
-B	1.63	1.89			12.70
-C	1.61	1.87			12.58
MA973-4-A	1.60	1.92	0.835	0.669	12.82
-B	1.68	2.01			13.44
-C	1.58	1.89			12.66

Table 4: Nicotine Data for Request Code MA974

<u>Sample Code</u>	<u>Wt % Nicotine</u>			Average Weight (g) filler per cigarette	<u>Mg Nicotine per Cigarette</u>
	Not Corrected for Recovery	Corrected for Recovery*	Average Recovery Factor*		Corrected for Recovery*
MA974-1-A	1.61	1.75	0.920	0.743	13.02
-B	1.62	1.76			13.05
-C	1.59	1.73			12.82
MA974-2-A	1.62	1.75	0.925	0.723	12.62
-B	1.66	1.79			12.95
-C	1.63	1.76			12.75
MA974-3-A	1.60	1.72	0.933	0.672	11.54
-B	1.57	1.69			11.34
-C	1.59	1.71			11.48
MA974-4-A	1.59	1.74	0.912	0.654	11.41
-B	1.62	1.77			11.58
-C	1.60	1.75			11.46

The Wt % Nicotine (Not Corrected for Recovery) values were the results reported by the Hewlett-Packard (HP) ChemStation Software (Version A.04.02) which collected and analyzed the output from the HP 5890 gas chromatograph. It is these results which are stored in the ARD LIMS and are printed out on a Requester Report. The Wt % Nicotine (Corrected for Recovery*) values were calculated using the equation:

$$\text{Wt \% Nicotine (Corrected for Recovery*)} = \frac{\text{Wt \% Nicotine (Not Corrected for Recovery)}}{\text{Average Recovery Factor*}} \quad (\text{Eq. 1})$$

As an example, consider sample MA974-4-B with a Wt % Nicotine (Not Corrected for Recovery) of 1.62 and a Recovery Factor of 0.912:

$$\text{Wt \% Nicotine (Corrected for Recovery*)} = \frac{1.62}{0.912} = 1.77$$

Mg Nicotine per Cigarette (Corrected for Recovery*) values are calculated using the following equation. Data on the average weight of filler per cigarette has been provided by PTL.²

$$\text{Mg Nicotine per Cigarette (Corrected for Recovery*)} = \frac{\text{Wt \% Nicotine (Corrected for Recovery*)}}{100} \times \text{Average Weight (g) filler per cigarette} \times 1000 \quad (\text{Eq. 2})$$

As an example, consider sample MA974-4-B with a Wt % Nicotine (Corrected for Recovery*) of 1.77 and an average weight of filler per cigarette of 0.654g:

$$\text{Mg Nicotine per Cigarette} = \frac{1.77}{100} \times 0.654 \times 1000 = 11.58.$$

(Corrected for Recovery*)

Since the Wt % Nicotine value represents the grams of nicotine per 100 grams of tobacco sample, the 1.77 value is divided by 100 to convert to grams of nicotine per gram of tobacco sample. The factor of 1000 is incorporated into the equation to convert grams to milligrams.

References:

1. Federal Register, Vol. 62, No. 85, May 2, 1997.
2. Product Testing Laboratory Procedures Manual, PPI 095-340, March 01, 1995.
3. W. McCoy, PM Notebooks 9345 and 9472.
4. I. Skinner, PM Notebook 9461.

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* The specified CDC protocol cited in the Federal Register/Vol.62, No.85, 24115 through 24119, Standards Addition Assay, II.B.1. through 12., was followed as written and results reported with specified recovery correction, II.D.8. In II.B.2., instructions and calculations related to the nicotine and internal standard concentrations are in error.